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Remediation of a Freshwater Wetland in the Presence and Absence of Wetland Plants Through Enhanced Biostimulation. Part I: Effectiveness

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When oil spills occur, especially ones involving supertankers where huge amounts of oil are discharged into the environment, the environmental damage can be enormous. Not only is the affected area aesthetically ruined by the unsightliness of the tarry, black residue, but more importantly, the effects on wildlife and aquatic animals are often devastating. Local ecological diversity can be seriously threatened and food chain interactions significantly disrupted for years following the catastrophe. The reason why it is so essential to accelerate cleanup is to prevent or mitigate further damage not only to exposed living populations but also to humans who eventually consume animals that may have concentrated the contaminating compounds through trophic level biomagnification. Many compounds in crude oil are environmentally benign, but a significant fraction are toxigenic or mutagenic. The latter are the ones we are most interested in removing or destroying. Bioremediation and phytoremediation are technologies that offer great promise in converting the toxigenic compounds to nontoxic products without further disruption to the local environment. This project was undertaken to develop an understanding of how to implement bioremediation for cleanup of a catastrophic spill on the ecologically, environmentally, and economically important St. Lawrence River. The project is sponsored by the U.S. Environmental Protection Agency and Fisheries and Oceans Canada.

One of the main challenges associated with biostimulation (i.e., nutrient enrichment to enhance bioremediation) in oil-contaminated coastal areas or tidally influenced freshwater rivers and streams is maintaining optimal nutrient concentrations in contact with the oil. Oil from offshore spills usually contaminates the intertidal zone of marine beaches or the near shore zone of rivers, where the washout rate for water-soluble nutrients can be very high. Many attempts have been made to design nutrient delivery systems that overcome the washout problems characteristic of intertidal environments. These include oleophilic and slow-release fertilizer formulations and systems that rely on the subsurface flow of water through the beach. The effectiveness of these various technologies has not been convincingly demonstrated under field conditions, however, and it is likely that the most effective approach will depend on the characteristics of the contaminated environment.

Phytoremediation, the use of vegetation for the *in-situ* treatment of contaminated soil and sediment, is an emerging technology that has been shown to be an effective and inexpensive cleanup option for certain hazardous wastes. Phytoremediation technology has already been shown effective for the removal of both inorganic and organic pollutants, including polycyclic aromatic hydrocarbons (PAHs). However, little research has been conducted to assess the capacity of revegetation to enhance the biodegradation of hydrocarbons in freshwater wetlands. Phytoremediation may prove particularly effective when used in conjunction with biostimulation because the addition of agents such as fertilizers that enhance indigenous microbial activity and hence hydrocarbon degradation rate will also stimulate plant biomass production and thereby increase the effectiveness of phytoremediation.

The objectives of the field study were: (1) to determine the effectiveness of bioremediation with and without the confounding effects of wetland plants to restore the contaminated area to pre-spill conditions; (2) to determine the effect of transplanting fresh plants into the impact zone (phytoremediation) to restore the impacted ecosystem; and (3) to evaluate the ecotoxicity of the oil exposure and the subsequent bioremediation/phytoremediation activities and the accompanying reduction in toxicity as restoration takes place.

The experimental area consisted of 20 plots each measuring 5 m x 4 m in area, with the 5 m dimension parallel to the shoreline. Each plot was boomed to safeguard the area from an accidental release of crude oil into the surrounding environment. The five treatments included a no oil control and four oiled treatments. The oiled treatments included a natural attenuation control (no amendments), a treatment receiving granular ammonium nitrate and triple super phosphate tilled into the top 2-5 cm of sediment, a treatment receiving granular ammonium nitrate and triple super phosphate tilled similarly but with the wetland plants cut back to ground surface to prevent growth, and a treatment receiving sodium nitrate rather than ammonium nitrate tilled into the sediment.

The crude oil (Mesa light crude) was applied during the week of June 7, 1999 at a rate of 12 L per plot. Sampling began one week after oiling on the day when the first nutrient application was made. The subsequent sampling intervals were at weeks 1, 2, 4, 6, 8, 12, 16, 21, and one to be conducted this spring, yielding a total of 10 sampling events.

A stratified sampling plan was developed resulting in two composite samples at each sampling event that were representative of the entire plot. The composites were analyzed for oil composition analysis by GC/MS (analytes were normalized to hopane) and for measurement of ecotoxicity and microbial populations (alkane and PAH degraders by MPN analysis). Nutrients (NH_4^+ , NO_3^- , and PO_4^{3-}) were monitored twice weekly. If the nitrogen fell below about 5-10 mg/L in the interstitial pore water, more fertilizer was added to the surface, but the sediment was not tilled. Other analyses performed included solid-phase and liquid-phase MicrotoxTM, cell line bioassays (mixed function oxidases), amphipod mortality, and accumulation of hydrocarbons using Semi-Permeable Membrane Devices (SPMDs).

Preliminary results from the GC/MS analysis indicated slow but steady biodegradation occurred in all plots through the 21-week sampling period. Although the results have not been subjected to statistical analysis yet, the data suggest that very little treatment effects were noticeable. In other words, the biodegradation that was occurring appeared not to be enhanced by fertilizer addition. However, at and subsequent to week 12, in addition to the regular core samples, additional samples were collected from the top cm surface of each plot. These samples revealed that an enhanced biodegradative removal occurred in the plots with cut plants that had received ammonium as the nitrogen source. The preliminary conclusion is that, if oil contaminating a wetland is able to penetrate below the surface substantially, oxygen becomes limiting to the oil degraders even if substantial nutrient concentrations exist in the sediment. The wetland plants are unable to pump sufficiently more oxygen into the rhizosphere to aid the soil microorganisms to break down the oil. At the surface, enough oxygen is present to enable accelerated bioremediation to take place in a thin surficial zone. In addition, the existence of living plants appears to inhibit biodegradation, presumably because the plants are able to out-compete the sediment oil degraders for available nutrients. These conclusions are preliminary, however, and it is cautioned that they should not be seriously considered as final until a thorough analysis of all the data has been made.

Remediation of a Freshwater Wetland in the Presence and Absence of Wetland Plants through
Enhanced Biostimulation. Part II: Habitat Recovery and Toxicity Reduction

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The St. Lawrence River/Seaway is one of the most difficult waterways to navigate due to the presence of strong currents and tides. Nevertheless, each year more ships pass through its waters than the Panama and Suez canals combined. This system plays an important role for the distribution of North America's energy reserves; in 1988, 10.8 million tonnes of refined petroleum products and 6.9 million tonnes of crude oil were transported on this waterway by tankers having a holding capacity as high as 160,000 tonnes. With such a heavy volume of marine traffic, the risk of a major shipping incident is high. Wetlands along the St. Lawrence are important ecologically and economically, as they provide the principal nursery ground for coastal fisheries, habitat for wildlife and endangered species, and protection against shoreline erosion. Indeed with the identification of more than 140 rare species, some wetland reserves in the region have been declared "world heritage sites".

Information on impact of oil spills in north-temperate freshwater wetland ecosystems is limited. In addition to the need to understand the potential environmental impact of oil spills on wetlands, there is a need to develop effective habitat restoration technologies. To address these issues, Fisheries and Oceans Canada and the US EPA have sponsored a joint project to refine and validate biostimulation strategies such as bioremediation and phytoremediation to accelerate habitat recovery.

The public has responded favourably to biostimulation as an operational oil spill countermeasure, as its implicit goal is that of reducing toxic effects by converting organic molecules to benign cell biomass and "environmentally friendly" products such as carbon dioxide and water. However, a fraction of the oil spill response community, environmentalists, and the public remain concerned over the net benefit of such countermeasures. Concerns include the potential production of toxic metabolic by-products, possible toxic components in the formulation of bioremediation agents, and the incomplete degradation of highly toxic components within the residual oil. These environmental issues and a direct measure of remediation treatment success can be directly addressed by the application of ecotoxicological monitoring techniques.

An experimental oil spill experiment was conducted in the summer of 1999 at a wetland site situated along the St. Lawrence River, at St. Croix, Quebec, Canada. The study area consisted of 20 experimental plots (5 m x 4 m) dominated by *Scirpus pungens*. Experimental treatments included an uniled control and 4 oiled treatments. The oiled treatments included a natural attenuation (no treatment) control, nutrient amendment with granular ammonium nitrate and super triple phosphate, a similar treatment with plants continuously cut back to suppress plant growth, and a treatment consisting of amendments with sodium nitrate rather than ammonium nitrate. Weathered Mesa light crude oil was applied during the week of June 7, 1999 at the rate of 12 L per plot. To ensure that nutrient limitation was not a factor suppressing treatment effects, nutrients were reapplied when interstitial nitrogen concentrations fell below 5-10 mg/L.

Time-series sampling for chemical and toxicological analysis was initiated one week after oiling concurrent with the first nutrient application event. Samples were recovered over a

21-week period that effectively covered the natural growth season of the plants. The extent oil biodegradation was quantified by GC/MS analysis with analytes normalized to the conserved marker hopane.

Impacts and habitat recovery was assessed by monitoring the effect of oil on the growth and survival of *Scirpus pungens* and the reduction of sediment/interstitial water toxicity within a selected battery of toxicity tests. Regulatory biotests included the Microtox Solid Phase Test, the *Hyalella azteca* 14-day Growth and Survival test, the 48-hour *Daphnia magna* Survival Test, and the Algal Solid Phase Assay (ASPA). The indigenous prosobranch gastropod, *Viviparus georgianus* was also used as a biomonitor to assess the direct impact of oil on the survival, growth and reproduction of benthic invertebrates within the test plots. Initial results have demonstrated the tolerance of *Scirpus pungens* to the concentration of oil applied in during this experimental spill. While nutrient additions clearly enhanced the growth of the wetland vegetation in both oiled and unoiled test plots, results from the Microtox Test suggest that reduction of sediment toxicity did not occur at a rate higher than that found in the oiled control (natural attenuation). The results of this preliminary toxicity data set are supported by the GC/MS data presented in the previous paper, which suggest that there was little treatment effect. This conclusion based on the result of the Microtox Test results is preliminary, as an accurate environmental assessment cannot be made on the result of a single biotest. Conclusive remarks can only be made when all toxicological, chemical, and microbiological data have been interpreted.